Please replace the paragraphs at page 20, line 13, to page 21, line 24, with the following paragraphs:

Retinoblastoma (Rb) gene involves chromosomal abnormality on chromosome 13 q 14 (the long arm). Retinoblastoma is a malignant abnormality that results from the loss of the Rb gene. Although this gene is normally found in 2 copies (one on each chromosome 13), certain individuals will have one or both copies of the Rb gene deleted. If both copies of the gene are deleted, the onset of malignancy happens rapidly. If one copy is deleted, then a secondary mutation event is required before malignancy occurs. The gene product is involved with regulation of cell proliferation and its loss eliminates the tight controls placed on cell division. In addition to retinoblastoma, a deleted or dysfunctional Rb gene has been associated with osteosarcomas and solid tumors in other organs.

There are numerous other genetic disorders and genes that lend themselves to the gene therapy protocols proposed in this invention. While the number of genes that could be altered by SFHR is large, a primary requirement will be knowledge of the DNA sequence that constitutes a given gene. When this information is known or obtained, the invention provides straight-forward method to design a SFHR gene therapy strategy that encompasses both the generation of the incoming therapeutic DNA and the analysis of the target cells after SFHR. In particular, inherited diseases that are the result of small mutation (e.g., base changes, small deletions or insertions) are particularly amenable to gene therapy by SFHR. In addition to those diseases mentioned above, SFHR gene therapy could be applied to adenosine deaminase deficiency (ADA), Lesch Nyhan syndrome, Duchenne muscular dystrophy, and Fanconi's anemia, to mention a few. Furthermore, the SFHR gene therapy protocol could be applied to the treatment of certain infectious diseases.

In the treatment of infectious disease, the incoming exogenous DNA contains mutations that inactivate the pathogen by introducing these inactivating mutations into essential genes of the genome of the pathogen. Retroviral pathogens, such as HIV, the agents thought to be responsible for acquired immune deficiency syndrome (AIDS) and the hepatitis B virus are candidates for SFHR gene therapy of infectious diseases. These viruses rely on the integration of the proviral DNA into the cellular genome as part of their replicative cycle. The proviral DNA is the target for the incoming exogenous DNA fragments. A cocktail of fragments with inactivating mutations, that are homologous to several different essential genes within the viral genome is used to disrupt the

integrity of the viral genome and the viruses ability to replicate.

Please replace the paragraph at page 24, lines 25-29, with the following paragraph:

The normal DNA fragment whether uncoated, coated or complexed is preferably delivered into the cell complexed with a protein-lipid complex, complexed with a lipid layer or with a dendrimer or by electroporation or microinjection according to Examples 15-17.

Please replace the paragraph at page 25, lines 19-23, with the following paragraph:

Homologous replacement according to the invention has been tested, successfully achieved and a correction of a dysfunction in cystic fibrosis has been observed. Other diseases subject to the same treatment are sickle cell anemia and xeroderma pigmentosum group G.

Please replace the paragraph at page 39, lines 10-19, with the following paragraph:

Results in this study demonstrate that (a) .DELTA.F508 CF epithelial cells undergo homologous replacement at the CFTR mutation with small fragments of wtCFTR DNA, resulting in a corrected genomic .DELTA.F508 locus; (b) rec A protein-coated and uncoated ssDNA fragments can be used in the transfection of cultured human cells; (c) cystic fibrosis .DELTA.F508 mutations corrected in genomic DNA result in production of normal CFTR mRNA; and (d) CF cells corrected by homologous replacement display intact cAMP-dependent Cl transport.

Please replace the paragraph at page 40, line 26, to page 41, line 20, with the following paragraph:

The patch clamp analysis shows that within a population of transfected CF cells there appears to be a significant subpopulation cells in which cAMP-dependent Cl transport defect has

been corrected. In these experiments precautions were taken to reduce the contribution of K.sup.+ currents to the whole cell current by having CsCl in the pipette filling solution. The continuous recording of cell membrane potential kept the cells at their own membrane potential and reduced artifacts due to cell swelling or voltage clamping. The increase in whole cell current in 7 of 78 forskolin treated cells and the depolarization due to the reduction of the bath Cl in each of the 7 responding cells, clearly shows that the current increase in the whole cell was due to activation of a Cl conductance. The apparent homologous replacement frequency of 9% is a maximum frequency in that it assumes that each responding cell represents a single homologous replacement event. However, the possibility that in cases where multiple responding cells were detected (GS- and SD+, Table 2), each responding cell may have been derived from a single cell in which homologous replacement occurred can not be ruled out. If this was the case, the responding cells would, in effect, reflect 3 separate homologous replacement events and would indicate a frequency of -4% (3 in 74 cells). These values show close agreement with the calculation of frequency from the densitometric analysis of homologous replacement frequency (3-7%). In any event, the degree of homologous replacement and phenotypic correction is well within the range of 6-10% that appears to be sufficient for conversion of a CF epithelial monolayer to one with normal cAMP-dependent Cl transport properties as reported in Nat. Genetics, 2:2 (1992).

Please replace the paragraphs at page 48, lines 13-28, with the following paragraphs:

DNA samples from the T6/20 plasmid (lanes 1, 4, and 7), nonCF lymphocytes (lanes 2, 5, and 8), and CF airway epithelial cells, ΣCFTE29o- (lanes 3, 6, and 9). No PCR products were detected when one primer based on the previously published sequence was used (lanes 1-3). Primers based on sequence data reported here were able to show PCR products from all DNA samples (lanes 4-9).

An expected 485-bp fragment was detected using primer CF39 and C16D in DNA samples isolated from non-CF human lymphocytes, ΣCFTE29o-, a trachea epithelial cell line derived from a CF patient homozygous for the ΔF508 mutation and from the plasmid T6/20 seen in FIG. 17. Similarly, PCR amplification with primers CF40/CF41, localized outside previously published sequence, was able to generate the expected 1057-bp fragment from the three DNA samples

indicated above as seen in FIG. 17.

Please replace the paragraph at page 54, line 34, to page 55, line 4, with the following paragraph:

The normal DNA fragment is delivered into the cells under conditions effective for homologous replacement to occur. The resulting genetically altered cells can then be delivered to the subject in need of gene correction. This ex vivo alteration of cells and their transplantation into an afflicted individual comprises another mode of delivery of the exogenous DNA fragment, as opposed to direct introduction of the fragments in vivo.

Please replace the paragraph at page 67, lines 6-11, with the following paragraph:

The β-gal reporter system indicates correction by the appearance of blue cells following X-gal treatment. Only the cells with a functional β-galactosidase gene are blue. The fraction of blue cells within the population of transfected cells is readily determined by cell counting.

Please replace the paragraph at page 70, lines 22-31, with the following paragraph:

In addition, the above compounds according to the invention and their pharmaceutically acceptable derivatives may be employed in combination with other therapeutic agents for the treatment of the indicated conditions. Examples of such further therapeutic agents include agents that are effective for treatment of associated conditions. However, other agents that contribute to the treatment of the disease and/or its symptoms, such as inhibitors of neutrophil function, retinoic acid, anti-inflammatory agents, adenosine agonists, and the like, are suitable.

Please replace the paragraph at page 71, line 33, to page 72, line 2, with the following paragraph:

The delivery of the normal DNA fragment into the cell may be conducted by a variety of techniques discussed above. These encompass providing the altering DNA fragment enveloped by a lipid layer, complexed with a protein and a lipid or a dendrimer. The conditions for contacting any of these compositions with the cells to be altered were described above.

Please replace the paragraph at page 74, line 12, to page 75, line 7, with the following paragraph:

The cell recognition element is a molecule capable of recognizing a component on the surface of a targeted cell, covalently linked with a DNA-associating moiety by conventional methods. Cell recognition components include antibodies to cell surface antigens, ligands for cell surface receptors including those involved in receptor-mediated endocytosis, peptide hormones, etc. Specific ligands contemplated by this invention include carbohydrate ligands such as galactose, mannose, mannosyl 5-phosphate, fucose, sialic groups, N-acetylglucosamine or combinations of these groups as complex carbohydrates such as those found on glycolipids of the blood groups or on various secreted proteins. Other ligands include folate, biotin, various peptides that can interact with cell surface or intracellular receptors such as the chemoattractants peptide N-formyl-met-leu-phe, SEQ. ID. No.: 79 peptides containing the arg-asp-glycine sequence SEQ. ID. No.: 80 or cys-ser-gly-arg-glu-asp-val-trp SEQ. ID. NO.: 82 peptides, peptides that contain a cysteine residue or that interact with cell surface protein such as the human immunodeficiency virus GP-120, and peptides that interact with CD-4. Other ligands include antibodies or antibody fragments. The specificity of the antibodies can be directed against a variety of epitopes that can be expressed on cell surfaces including histocompatibility macromolecules, autoimmune antigens, viral, parasitic or bacterial proteins. Other protein ligands include hormones such as growth hormone and insulin or protein growth factors such as GM-CSF, G-CSF, erythropoietin, epidermal growth factor, basic and acidic fibroblast growth factor and the like. Other protein ligands would include various cytokines that work through cell surface receptors such as interleukin 2, interleukin 1, tumor necrosis factor and suitable peptide fragments from such macromolecules.

Please replace the paragraph at page 77, lines 13-31, with the following paragraph:

The direct administration of small ssDNA fragments advances previous homologous replacement because it shows that homologous replacement with small genomic DNA fragments is successful to correct naturally occurring genomic CFTR mutations in CF epithelial cells, in sickle cells and skin cells. In addition, SFHR has an advantage over vector based homologous recombination strategies reported before in Nature, 346:847 (1990) because intron sequences are not disrupted by selectable marker gene sequences. This eliminates the possibility of interference of marker gene transcription with that of the targeted gene. The SFHR approach also presents an advantage over cDNA gene therapy strategies, because the corrected gene continues to be regulated by endogenous genomic enhancers and promoters rather than a heterologous enhancer and promoter in the vector. Thus, homologous replacement increases the probability that the corrected gene, whether CFTR or another gene, is expressed in the appropriate cells at the appropriate levels.

Please replace the paragraph at page 81, lines 20-32, with the following paragraph:

The cells were then washed with 2 changes of PN buffer for 3 min at room temperature and incubated with biotinylated anti-avidin (5 μg in PNM solution) at room temperature for 20 min under a coverslip. The slides were washed with two changes of PN buffer, 3 min. each, at room temperature and the cells stained with FITC-avidin (5 μg/ml in PNM solution) at room temperature for 20 min under a glass coverslip. The slides were again washed 2 times, 3 min each, with PN buffer at room temperature. Propidium iodide (2 μg/ml) was then added and the slides were viewed by fluorescence microscopy. The number of copies of chromosome 7 per cell was determined by counting fluorescent dots per nucleus.

Please replace the paragraph at page 90, lines 15-26, with the following paragraph:

The 491 bp fragment was prepared as described, denatured by heating to 95°C. for 10 min and then rapidly cooled on ice. 5 µl of the DNA were then added to a buffer solution. The buffer contained 20 mM Tris acetate, 10 mM Mg acetate, 70 mM potassium acetate, 1 mM dithiothreitol, and 100 µg/ml bovine serum albumin-fraction V, 0.5 mM ATP-γ-S followed by the addition of UvsX: 20 µl of a 1.4 mg/ml solution according to J. Biol. Chem., 261:6107-6118 (1985); UvsY: 36 µl of a 0.5 mg/ml solution J. Biol. Chem., 268:15096-15103(1990); and T4 G p32: 100 µl of a 5.2 mg/ml solution according to J. Biol. Chem., 263:9427-9436(1987) to a final volume of 250 µl.

D14

Please replace the paragraph at page 96, lines 6-18, with the following paragraph:

Electroporation experiments were performed using recombinase-coated 491-mer ssDNA as described above. Approximately 10^7 exponentially growing cells were suspended in 400 µl of recombinase coating buffer with 5 µg (5 µl) of recombinase-coated DNA. The cell suspension was preincubated on ice for 10 mins. and electroporated at 4°C. with 400 V and 400 µF in a BTX 300 electroporator (BTX Corporation, San Diego, Calif.). After electroporation, cells were incubated on ice for an additional 10 mins., diluted in Eagle's minimal essential medium (MEM) supplemented as described above, then seeded in a T75 flask. Under these electroporation conditions, approximately 30-50% of the cells survive. Cells were cultured at 37°C. in a humidified CO_2 incubator for 5-7 days and then harvested for DNA and RNA.

D15